Intranasal Curcumin Attenuates Silicosis by Inhibiting Matrix Metalloproteinase-9 (MMP-9) Activity in Mice

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Research Article

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Abstract

Inhalation of crystalline silica causes silicosis, a type of occupational pulmonary fibrosis, is most prevalent among people working in industries related to construction.Curcumin being an antioxidant and anti-inflammatory in nature has shown anti-asthmatic effectsbut it has not yet been investigated to have any impact on silicosis. Therefore,our aim was to study impact of intranasal curcumin on lung fibrosis after sequentialsilica exposure. The mice model of silicosis was developed by intranasal silica instillation(2.5 mg/mice) for every alternate dayfor different durations, mainly 7, 14 and 21 days. This model of silicosis mimic chronic occupational exposure of silica dustand severe features of silicosis were developed in 21 days of silica exposure.One hour prior to silica administration, curcumin (5 mg/kg,i.n) and /or dexamethasone, a known corticosteroid (10 mg/kg,i.p)was administered in mice.Results haveshown that apart from being anti-inflammatory, curcumin is being reported here for the first time to possess anti-fibrotic effects where silica exposed airway inflammation and fibrosis was reduced after intranasal curcumin treatment. Reduced inflammatory cell recruitment, collagen deposition around the bronchioles and the alveolar spaces, hydroxyproline level and matrix metalloproteinases 9 (MMP 9) activity was noted in silicosis affected mice after curcumin administration.Remarkable reduction in oxidative stress markers like reactive oxygen species (ROS), nitric oxide, myeloperoxidases (MPO) and eosinophil peroxidase (EPO) levels wereobserved in curcumin treatment groupswhich was better and/or comparable to corticosteroid, dexamethasone.

1. Introduction

Silica is the 2nd most common metal on earth next to carbon. Two oxygen atoms with one silicon together forms silicon dioxide or silica that occurs naturally as quartz or sand. Inhalation of crystalline silica causes silicosis which is a type of pulmonary fibrosis disease and is most prevalent due to occupational exposure such as road construction, quarrying, stone cutting, sandblasting, rock drilling and pottery [1].

On the basis of duration of exposure, silicosis is divided into several subtypes: chronic silicosis, acute and accelerated silicosis. Chronic silicosis is developed by low concentration of exposure for 10 years whereas speed-up silicosis progresses after five to ten years of continuous to medium exposures. Higher concentrationsof silica exposures for three or four weeks to 5 years causes acute silicosis [2].It is difficult to mimic human silicosis in mice as disease development takeslonger time to develop butmice silicosis features resembled to that of humansilicosis [3, 4]. The mechanism of silicosis and related inflammatory response is still to be studied further to develop effective therapy for curing silicosis. Choices for treatment for chronic silicosis are currently very sparse and is further followed by complex unfavourable outcomes[2].

Inhalation of crystalline silica particles reaches the lungs where it induces oxidative stress by the formation of reactive oxygen (ROS) and nitrogen species (RNS) and after crystalline-silica fracturing generatessiloxil radicals [5]. These respirable particulates gets deposited in the lung and endocytosed by

alveolar macrophages (AM) and remain in the phagosomes [6]. However, silica being a particulatematter,its degradation is not possible, therefore may getpermeabilized via phagolysosomal membrane [7, 8].Various inflammatory cell recruitmentsmay cause acute inflammatory response leading to pulmonary fibrosisbut its exact cause is not clearly understood. Secretion of various inflammatory cells such as macrophages and lymphocytes, and secretion of several pro-inflammatory, fibrotic cytokines and chemokines are the results of silicosis [3].It is also related to proliferation of fibroblasts and epithelial cells to mesenchymal transition,where E-cadherin surfacemarkers present on epithelial cells are lostand express mesenchymal cells start expressing markers such as vimentin and α-smooth muscle actin (α-SMA) [9].Excess extracellular matrix deposition because of constant lung inflammation and collagen deposition which ultimately leads to fibrosis and further respiratory failure. Respirable air particulates of smaller size is cleared by alveolar macrophages and are later stored in lysosomes, these lysosomes forms phagolysosomes by fusing with phagosomes[6, 10]. The lysosomal enzymes try to degrade these phygocytosed particles. Regardless, alveolar macrophages is unable to clear silica particles making this disease incurable.

Bronchodilators/ cough medication is generally used for the treatment of silicosis. Other treatments for silicosis involves regular check-up for any kind of respiratory infections and to protect patients from continuous vulnerability to foreign particulate irritants. Corticosteroids, a class of steroid hormones,has been shown modulate inflammation. Chinese medicine has been using Alkaloid Tetrandrine historically for the treatment of pneumoconiosis, its curative use for silicosis [11]

Curcumin (diferuloylmethane),an active constituent of turmeric, *Curcuma longa*. It is being used for many years in food for its flavour and also as a preservative. Cancer, cystic fibrosis and various other respiratory diseases have also been observed to be cured by curcumin [12].Its significant medicinal properties has attracted much of the attention in recent times.

Apart from variety of biologic properties, its anti-inflammatory and anti-oxidantproperty is effective in modulating several transcription factor, adhesion molecules, cytokines and chemokines. It is effective against various respiratory diseases. Curcumin pretreatment has been shown to suppressthe expressions of matrix metalloproteinases-9 (MMP-9), alpha smooth muscle actin (α-SMA) and tissue inhibitors of metalloproteinase (TIMP-1) [13]. Intranasal curcumin has been reported to be effective in pulmonary fibrosis by modifying matrix metalloproteinase-9(MMP 9) activity[14].Various inflammatory cytokines such as IL-8, MIP-1α, MCP-1, IL-1α, and TNF-α has been reported to be produced by alveolar macrophages and in human peripheral blood monocytes as a result of inflammation[15].

2. Materials And Methods

2.1 Reagents

Silicon dioxide (0.5–10 μm), curcumin (diferuloylmethane), DCFDA (dichlorofluorescindiacetate), vanadium chloride (VCl_3), Gelatin, hydroxyproline and Glutaraldehyde Grade 1 used as an electron

microscope fixative (Sigma-Aldrich ,St Louis, MO, USA). Phosphotungstic Acid, Phosphomolybdic Acid, Acid Fuchsin were purchased from Sisco Research Laboratories (Mumbai, India) and Aniline Blue was purchased from Fisher scientific (Hampton, New Hampshire, United States).

2.2 Animals

Mice (Swiss strain, 25-30gram) were obtained from Animal facility of Banaras Hindu University, Varanasi, India and accustomed for two weeks under standard conditions oflight and dark cycle and were on lab chow diet with water *ad libitum*.Institutional Animal Ethical Committee, Banaras Hindu University, Varanasi, India has approved all the procedures of animal maintenance.

2.3 Preparation of silica particles

Crystalline silica ($cSiO_2$) particles was sterilised at 120°C overnight then sonicated for 15 min in 200 mg/ml in autoclaved saline solution. Prior to intranasal administration of silica suspension, it was sterilised. Equal volume of autoclaved sterile saline was administered in control mice.

2.4 Experimental design

All mice were arbitarily allocated into 5 groupswith 5 mice each: **Control** group (saline only), crystalline **Silica-induced** group ($cSiO_2$ only), **curcumin** group ($cSiO_2$ + Cur), **vehicle** ($cSiO_2$ + DMSO) group and **Dexa** group ($cSiO_{2+}$dexamethasone). Mice model of Silicosis was developed by intranasal administration of crystalline Silica ($cSiO_2$) for different durations, mainly **7, 14 and 21 days** (every alternate day) to mimic the silicosis in human. Each duration of silicosis (7, 14 and 21 days) was thoroughly investigated by evaluating lung histopathology and biochemical parameters and silicosis features were observed in mice of 21 days of silica exposure, therefore, this duration was finally selected for detailed study. Curcumin was dissolved in Dimethyl Sulphoxide (DMSO) and given 10µl to each mouse (10 mg/kg, i.n., 5 µl/ nostril) an hour prior to silica administration. Dexamethasone (5 mg/kg, i.p.) was used as standard drug.

2.5 BALF and lung sample collection

After 24 hours of last intranasal silica administration, blood was collected via retro orbital bleedingandserum was separated by centrifugation and stored (at -20°C)to study biochemical parameters. Lungs were washed thrice by using 1 ml of sterile phosphate-buffered saline (PBS) and bronchoalveolar fluid (BALF) was collected,washed (at 3000 rpm for 15 minutes at 4ºC) and supernatant was stored (-80°C) for further analysis. Cell pellet was used for total and differential cell count. The lung lobes were separated and washed in chilled phosphate buffer saline solution. Some part of the lung lobes were fixed with 10% Neutral buffer formalin and rest were stored (-80°C) for biochemical analysis.

2.6 Reactive oxygen determination in BALF cell suspension

Reactive oxygen species (ROS) was measured using method standardised previously [16]. BALF was washed with PBS thrice and the cell pellet was collected by centrifugation. DCFDA (10 mM) was added to

the BALF pellet with equal amount of cells, thereafter incubation in dark at 37ºC for half an hour. Microplate fluorescence reader was used to measure fluorescence at excitation (485 nm)and emission (530 nm)wavelengths. Fluorescence intensity in arbitrary units was presented as ROS level.

2.7 Nitrite level measurement

Nitrite/Nitrate level in serum was analysed using griess reagent with some modifications[17]. BALF supernatant (100 µl) was mixed with VCl_3(0.8 gm/ ml in 1 N HCl;100 µl), Then freshly prepared Griess reagent (100 µl of 1% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-Naphthyl) ethylenediaminedihydrochloride in water) was added. The reaction mixture was incubated at 37ºC for 30 min and absorbance was read at 540 nm.

2.8 Lung histology for airway inflammation and collagen deposition

Lungs were removed, washed in cold sterile PBS and then two lobes were fixed by using 10% neutral buffer formalin. The left lobe was fixed in glutaraldehyde for electron microscopy. Remaining lobes were stored (in -80ºC) for further biochemical analysis.After embedding in paraffinwax,lung sections(5 µm) were cut using microtome, stained with H&E for inflammatory cell infiltration. To study collagen deposition and fibrosis, masson's trichome stained lung sections were analyzed.

2.9 Transmission electron microscopy(TEM)

Immediately after sacrifice,lung tissue pieces(1 mm^3) were made and fixed in 2% glutaraldehyde at 4°C,washedin 0.1 M phosphate buffer, pH 7.4for transmission electron microscopy (TEM). The sample was then taken to AIRF, JawaharLal University, New Delhi, India for further analysis(TEM).

2.10 Malondialdehyde assay (MDA)

Malondialdehyde (MDA) level was measured in lung tissue homogenate by thiobarbituric acid active substances (TBARS) using previously described method[18].Lung tissue homogenate in potassium phosphate buffer (10 %; pH 7.4) was made and mixed with (8.1% SDS, 375 µl of 20 % acetic acid) and 8.1 % thiobarbituric acid. After boiling for 1 h, it wascooled at room temperature to get pink colour. Distilled water (250 µl) was added followed by pyridine and n-butanol (1.25 ml of 1:1 solution). Mixture was separated by centrifuging at 2000 rpm for 10min and two layers were obtained and the absorbance of upper layer was read at 532 nm, and MDA concentration was expressed (moles / milligram).

2.11 Eosinophil peroxidase (EPO) activity

Eosinophil peroxidase (EPO), an enzyme is stored in the granules of eosinophils and its activity was determined using previously described method [19]. Equal concentrations ofBALF (100 µl in PBS) and substrate solution (consisting of 0.1 mM O-phenylene-diamine-dihidrochloride, 0.1% of Triton X-100, 1 mM hydrogen peroxide in 0.05 M Tris–HCl pH maintained at 8.0, wastaken and further incubated for 30

min at 37°C. Sulphuric acid (50 µl, 1 M) was added to stop this reaction. Absorbance wasread at 490 nm using ELISA plate reader.

2.12 Myeloperoxidase (MPO) activity

Myeloperoxidase (MPO) is an enzymestored in the granulesof neutrophils. Infiltration of these cells as inflammation marker was analysed by Myeloperoxidase (MPO) activitywhich was quantified by previously described method with minor changes[20]. Lung homogenate was prepared in potassium phosphate buffer (50 mM;pH 6.0), containing 0.5 % cetyl trimethyl ammonium bromide (CTAB) centrifuged at 12,000 rpm for 30 min,frozen (-80°C) and thawed. This process of freezing and thawing was repeated (thrice). Supernatant (20 µl)was mixed with reaction mixture containing 0.167 mg/ml Odianisidine dihydrochloride (ODD) and 0.002 % hydrogen peroxide (H_2O_2) in 50 mM potassium phosphate buffer. Change in absorbance for every 20 minutes at 460 nm was noted to measure the MPO activity using micro plate reader and the unit was denoted as MPO units per milligram of tissue.

2.13 Hydroxyproline Determination in Lungs

Hydroxyproline, an amino acid is the precursor and the main component in collagen, a triple alpha helix. Collagen content was measured using hydroxyproline content measurement [21].Acid digestion with 12 N hydrogen chloride (HCl) of lung tissue collagen is a good biochemical index for collagen content measurement.A part of lungs were homogenised (10% w/v in phosphate buffer saline). Equal volume of lung homogenate sample was acid hydrolysed (12 N HCl at 120°C for 16–18 hours). Tissue homogenate supernatant (50 µl) was collected after centrifugation at 13000 rpm for 15 min at 4ºC, suspended in citrate-acetate buffer. Citrate buffer consists of glacial acetic acid (1.25%), sodium acetate (7.24%), and citric acid of 5 %, 7.24% and 3.4% of sodium hydroxide (NaOH) in distilled water (pH 6.0). Freshly prepared 1.4 % chloramine-T solution with 10% N-propanol was added to the sample and kept at room temperature for 20 min. After addition of freshly prepared Ehrlich's solution (4-dimethylaminobenzaldehyde dissolved in 18.6 ml of n- propanol and 7.8 ml of 70% perchloric acid), sample was heated (at 65°C for 15 min). Optical density was taken at 550 nm and concentration of Hydroxyproline present in total lung tissue was calculated in µg units.

2.14 Collagenase Activity determination using gelatin zymography

MMP-9 protease activity was measured using gelatin zymography in bronchoalveolar fluid (BALF). Gelatin (10 mg/ml) was dissolved in resolving gel (10% SDS-PAGE), BALF supernatant protein (50 µg) was loaded in wells and gel was run at 4°C. After washing the gel for thrice (10–15 minutes to remove the sodium dodecyl sulphate (SDS), in renaturing buffer containing 2.5% Triton X-100, gel was incubatedfor 48 h at 37°C and washed in incubation buffer containing 50 mM Tris–Hydrogen chloride, 50 mM Tris

base, 5 mM Calcium chloride, 0.2 M sodium chloride and 0.02% NaN_3 (pH 7.5). The gel was stained with Coomassie Brilliant Blue R 250 stain for 10–20 min and destained till clear white bands visible in the stained gel. ImageJ software was used to analyse bands via densitometry.

2.15 Statistical Analysis

ANOVA was used to measure significant changes between two or more than two groups' using Tukey's and post hoc test comparison. Level of significance was considered at $p < 0.05$ by using SPSS 16 software. The values are presented as the mean ± SEM.

3. Results

3.1 Standardization of Silica exposure protocol by light microscopy

Lung histopathology after H&E and masson's trichome staining revealed silicosis induction after 7,14 and 21 days of silica exposurewhere maximum inflammation, bronchoconstriction and collagen deposition was compared. 21 days of silica exposure resembled human silicosis.So, this duration was selected for detailed study[Figs. 2 and 3].

3.2 Inflammatory cells recruitment

Infiltration of inflammatory cells such as alveolar macrophages could be seen in silica induced lung sections.Large number of inflammatory cell recruitmentswhich were seen inGiemsa stained cytospun slides of silicosis groupwere significantlyreduced in the lung sections of curcumin and dexamethasone treated groups[Fig 4].

3.3Light Microscopy and Transmission Electron Microscopic examinations oflungs

Normal lung sections stained with H&E revealed large lumen area in bronchioles with few inflammatory cells whereassignificantly thick alveolar septa due to recruitement of inflammatory cells were seen in silicosis affected lung sections. Infiltration of large number of neutrophils, lymphocytes and macrophages were observed around the bronchial and alveolar interstitium of silicosis group. Apart from inflammatory cells, bronchoconstriction was also seen in the silicosis group. In contrast, reduced inflammation could be seen in curcumin and dexamethasone intervention for 21 days as recovered alveolar and peribronchiolar regions can be seen. These results indicated that intranasal curcumin is effective in silica-induced alveolar inflammation in mice[Fig. 5].

The collagen deposition (blue area) was significantly higher near bronchiolar region and alveolar septa in silicosis lungsrevealed after Masson's trichrome stained lung sections. However, curcumin treatment reduced inflammation as well asdeposition of collagen fibres which was also correlated with hydroxyproline level in lungs[Fig. 6].Alveolar macrophages in lung sections of silicosis contain crystals, possibly haemozoin. TEM images of silica induced lungs showed thickened alveolar septa and deposition of collagen around alveoli which could be correlated with Masson's Trichromestained lung tissue sections via light microscopy [Fig. 7]. The alveolar septa were filled with congested capillaries, leukocytes and RBCs as observed by light microscopy in silicosis lungs.

3.4 Curcumin inhibits MMP-9proteinase (collagenase) activity in BALF

Matrix metalloproteinases (MMP-9), a 92 KDa type 4 collagenase is an extracellular matrix degrading enzyme which is also known for its important role in fibrosis by releasing fibroblast growth factors and vascular endothelial growth factors.Enhanced MMP-9 proteinase activity wasseen asmaximum gelatin digestion in silica exposed BALF samples were notedthan controlgroup. Intranasal curcumin treatment has reduced MMP-9 activity. Higher hydroxyproline levels could be correlated with high MMP-9 activity insilicosismodel whereas curcumin and dexamethasone treatment has significantly decreased the hydroxyproline content [Figs. 8 and 9].

3.5 Curcumin reduces oxidative damage caused by Silicosis

Intracellular reactive oxygen species (ROS) was significantly higher in intranasal silica group which was significantly reduced after intranasal curcumin treatment. Nitrite level in BALF was also higher in silica group which can be correlated with accumulation of various inflammatory cells and high oxidative damage. Intranasal curcumin pretreatment has reduced and nitrite level in BALF supernatant, hence decreasing the oxidative damage caused by the aggregation of inflammatory cells[Figs. 10 and 11].

3.6 Curcumin represses eosinophils infiltration

Eosinophil peroxidase (EPO), a heme peroxidase is an enzyme stored in secretory granules of eosinophils. EPO activity as marker of eosinophil recruitment and activation, was measured using the lung homogenate which was found higher in silicosis group than the control group($p > 0.05$), whereas curcumin and dexamethasone treatment has reduced the EPO level ($p > 0.05$) [Fig. 12].

3.7 Curcumin supresses Myeloperoxidases (MPO) activity

Myeloperoxidases, a lysosomal enzyme is presentin granules ofneutrophil and is released in the extracellular matrix during degranulation. Significantly higher MPO activity was noted in silicosis group with respect to control group. Curcumin and dexamethasone group showed decrease in myeloperoxidase level ($p > 0.05$) [Fig. 13].

4. Discussion

Long duration exposures at lower concentrations had higher risk for silicosis than those who worked for shorter duration at high concentration level [22]. Crystalline silica deposition in the lung parenchyma induces interstitial fibrosis which leads to deterioration of lung function and also results in respiratory illness symptoms.

Presently available medications include bronchodilators andrespiratory infections are requiredto monitor closely. Corticosteroid are known to reduce inflammator symptoms such as short-term treatment, but its long-term positive effects has yet not been proven causing increased risk of infections.

Curcumin, a natural herbal drug has also been reported to have significant anti fibrotic and anti-inflammatory properties in mice model of chronic asthma[14].In present study, murine model of silicosis was developed by intranasal silicainstillationfor different durations, mainly 7, 14 and 21days onevery alternate day and silicosis was induced after 21 days of silica exposure resembled to that of human and fibrosis factors such as increased deposition of collagen around bronchioles and alveolar spaces (blue bands in masson's trichome stained sections), and hydroxyproline, one of the major constituents of collagen was also elevated.

Macrophages play very important role in fibrosis disposition in silicosis which leads to shortness of breath, the main symptom of silicosis. We found enhanced cellular infiltration, specifically macrophages in giemsa stainedBALF cell pellet, which was reduced in intranasal curcumin and dexamethasone treatment groups [Fig. 4].Lung histopathology has also revealed large number of inflammatory cell accumulation around bronchioles and alveolar spaces. Alveolar septa was thick in silicosis group whereas intranasal curcumin and dexamethasone (i.p) treatment groups have shownreduced alveolar septa thickening.

Enhanced nitric oxide and ROS levels were correlated with oxidative damage generated by phagocytic cells after silica exposure which has been reported earlier, our studies have also shown significant increase in oxidative stress as revealed by enhanced levels of ROS and nitric oxide level in silicosis model[Fig. 10 &11].Malondialdehyde (MDA),being one of the secondary products of lipid peroxidation,has been used as an oxidative stress marker. In present study, enhanced MDA level in silicosis group was significantly reduced afterintranasal curcumin pretreatment,whereas potential of dexamethasone to reduce MDA level, was less than intranasal curcumin[Fig. 14].

To assess collagen deposition and degradation as marker of fibrosis,MMP-9 activity was evaluated using extracellular matrix degradation by gelatin zymography. Excess ECM deposition was detected in silica induced fibrotic lung sections. MMP-9 activity was significantly higher in silicosisgroup which was significantly reduced indexamethasone and curcumin pre-treatment groups suggesting its effectiveness in decreasing collagen deposition.

5. Conclusion

So,intranasal curcuminpretreatment is effective in modulating occupational lung disease like silicosis, which is a type of pulmonary fibrosis. Curcumin, being anti-inflammatory molecule has reduced inflammation by inhibiting inflammatory cells recruitment, oxidative lung damage via regulating ROS and Nitrite levelsalong withdownregulation ofMMP-9 activity evaluated by gelatin zymography, collagen deposition and hydroxyproline content.

Declarations

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Contributions

Dr Rashmi Singh (Corresponding Author) has designed the study and analyzed the data, Sneha Kumari (Research Scholar) has performed the study and drafted the paper, D Dash has helped in data analysis.

Ethics Approval and Consent to Participate

Not applicable.

Patient Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

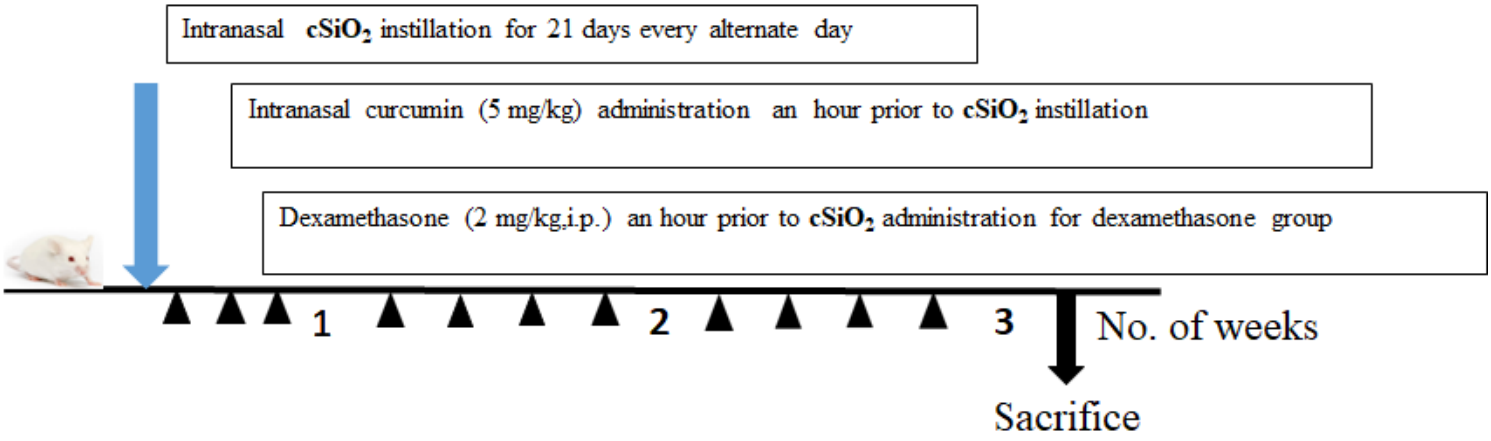

Fig 1

Figure 1

Experimental design: Five groups (n=5) were made; Control group (sterile saline ,i.n.), Silica group (intranasal cSiO2 for 21 days every alternate day), Curcumin group(5 mg/kg,i.n., 1 hour prior to cSiO2 instillation.), Dexamethasone group (2 mg/kg,i.p., an hour prior to cSiO2 administration).

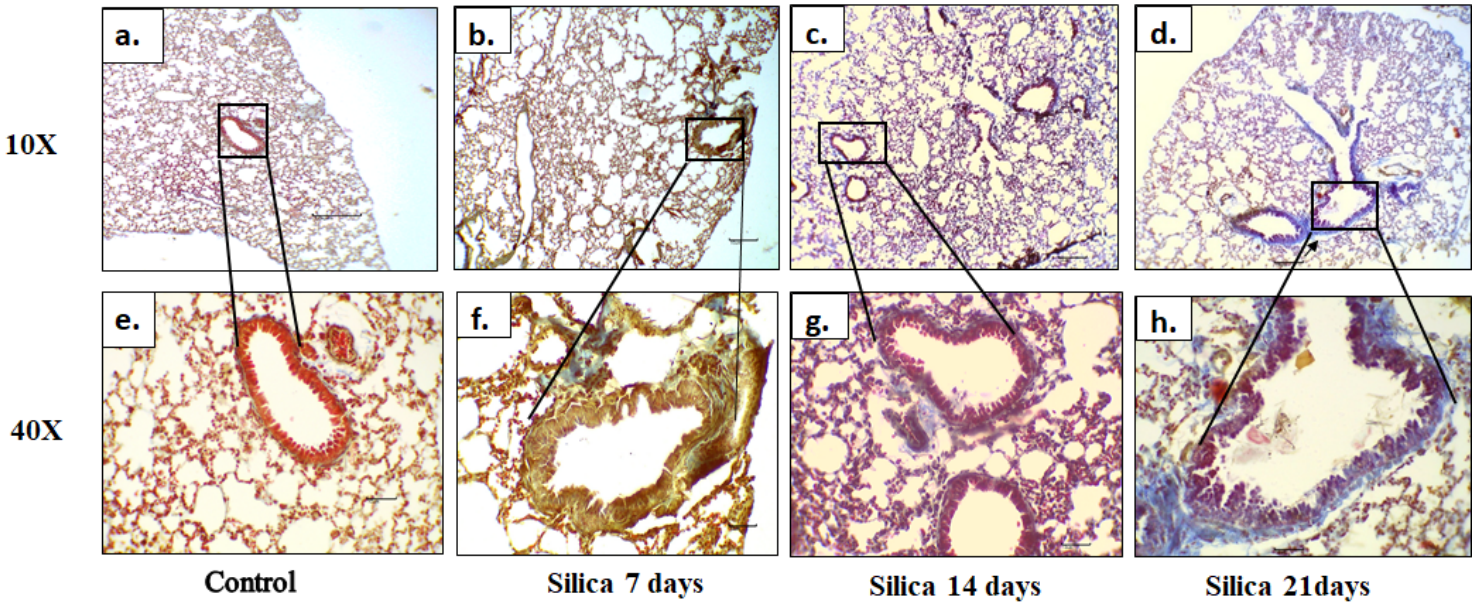

Fig 2

Figure 2

HE stained lung sections (5µm thin) after intranasal administration of cSiO2 every alternate day for 7days,14 and 21 days observed under light microscope at 10X and 40 X.

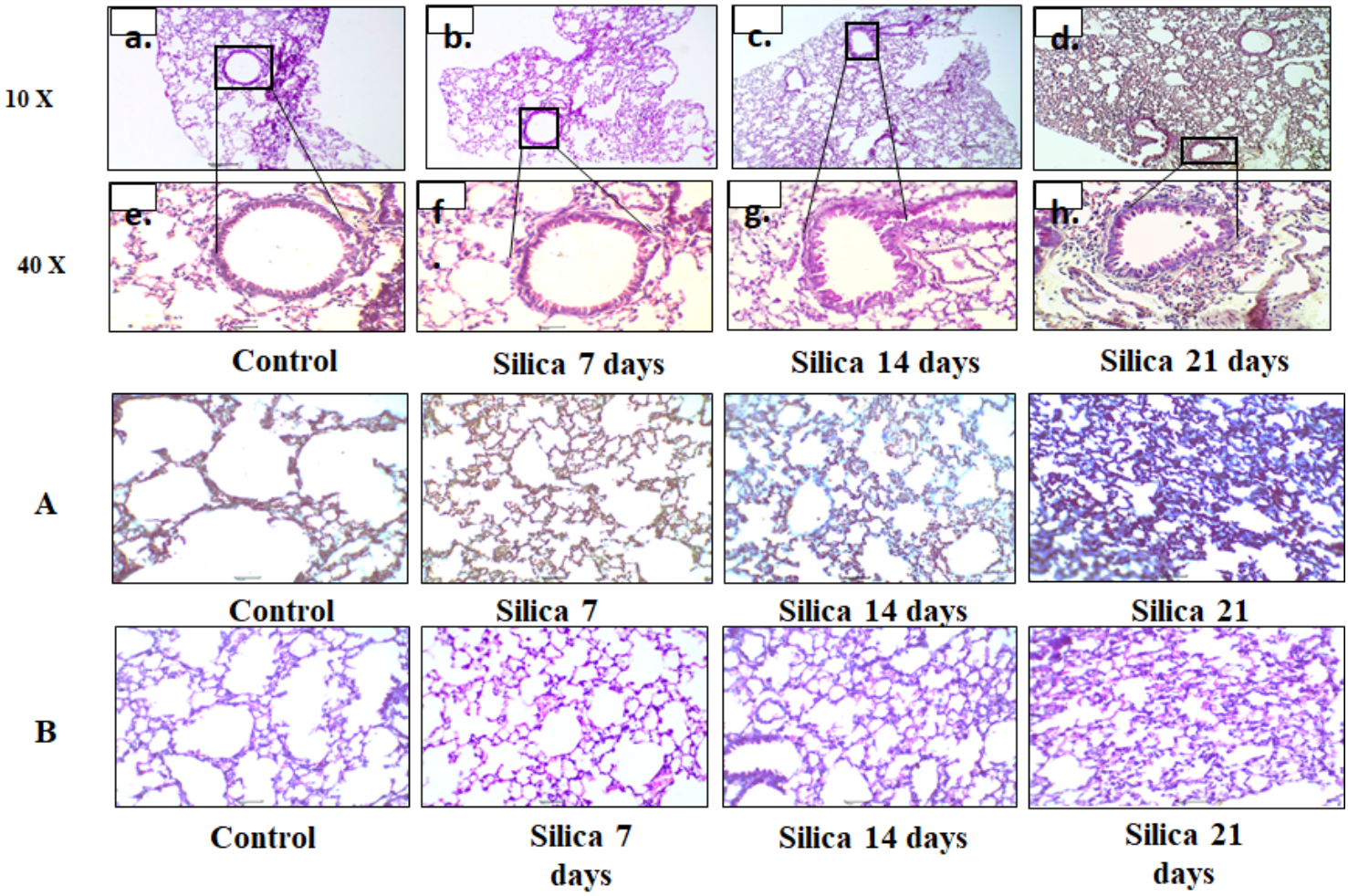

Fig 3

Figure 3

Alveolar spaces in lung sections of different model groups of cSiO2 exposure stained with Masson's trichrome [A] and H&E [B] [upper pannel : H& E stained sections and lower panel : A and B: Masson's trichome and H&E stained stained alveolar spaces]

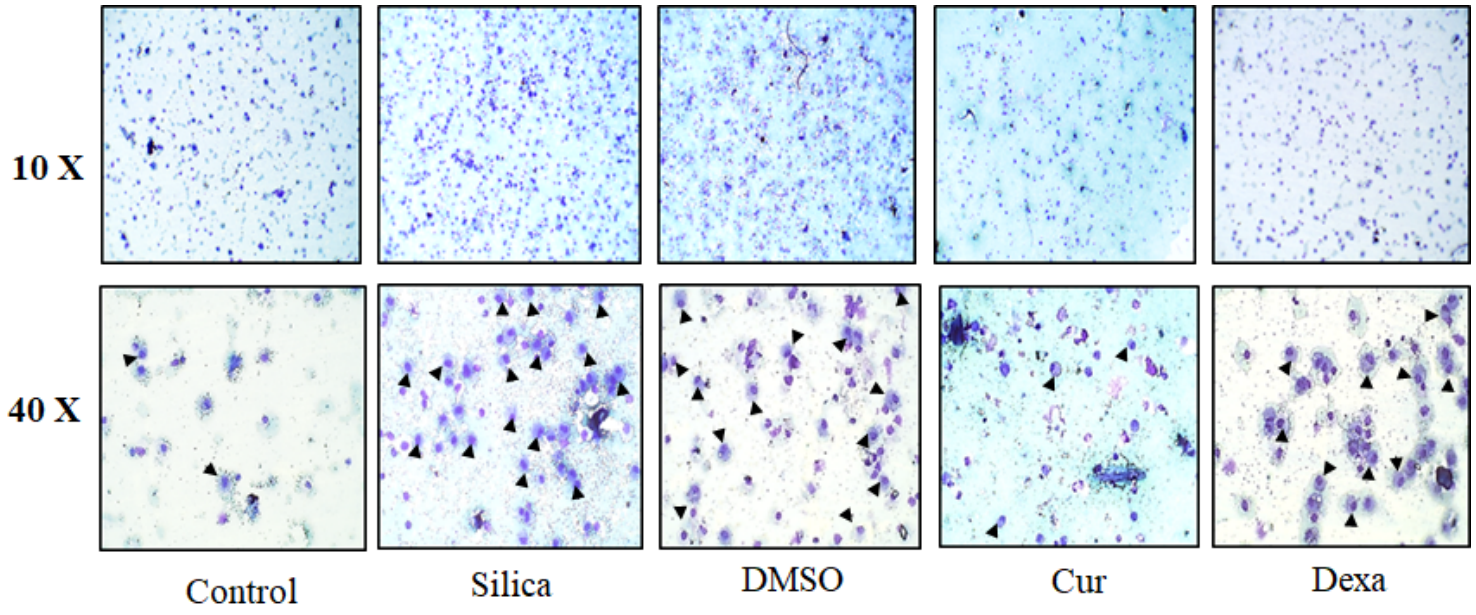

Fig 4

Figure 4

Cytospin preparation from BALF cell pellet. Enhanced Macrophage infiltration was seen in silicosis affected lungs whereas very few macrophages were seen in curcumin treated lungs. Interestingly, standard drug dexamethasone could not inhibit accumulation of macrophages like curcumin ($p<0.05$).

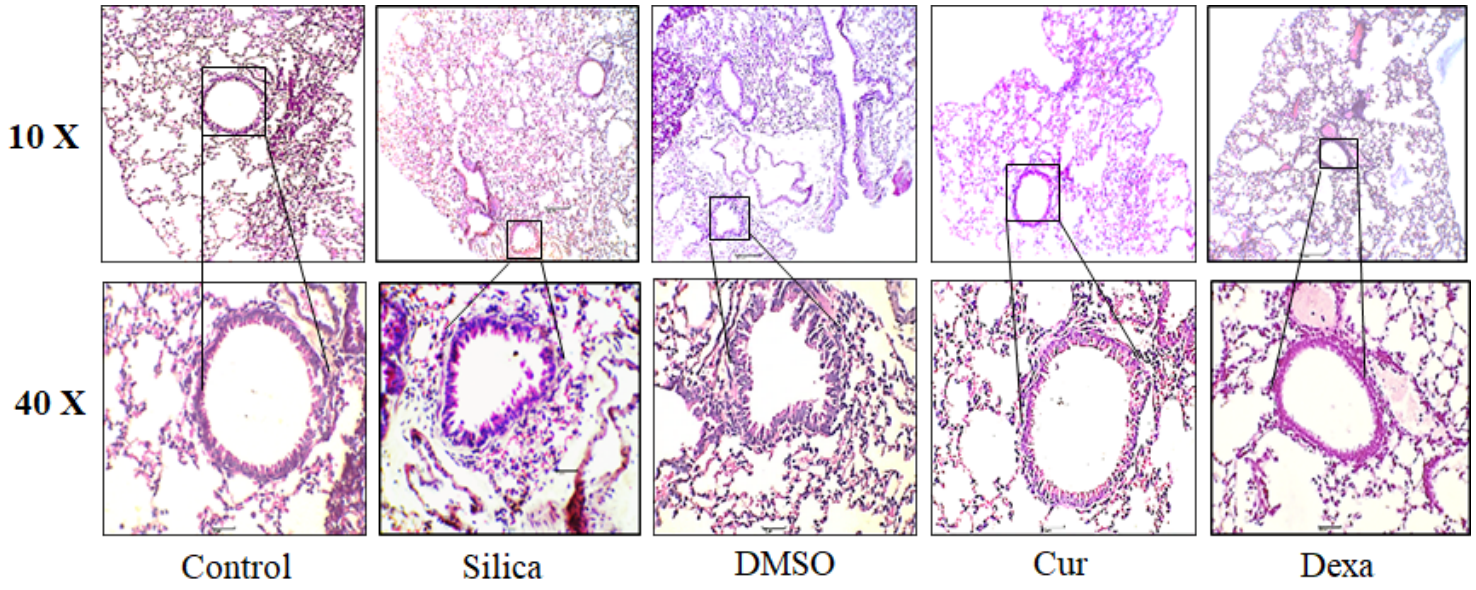

Fig 5

Figure 5

H&E stained lung sections. Bronchoconstriction and inflammation was noted in silicosis affected lung sections along with inflammatory cells around alveolar spaces.. Intranasal curcumin showed reduced inflammation better ithan dexamethasone group.

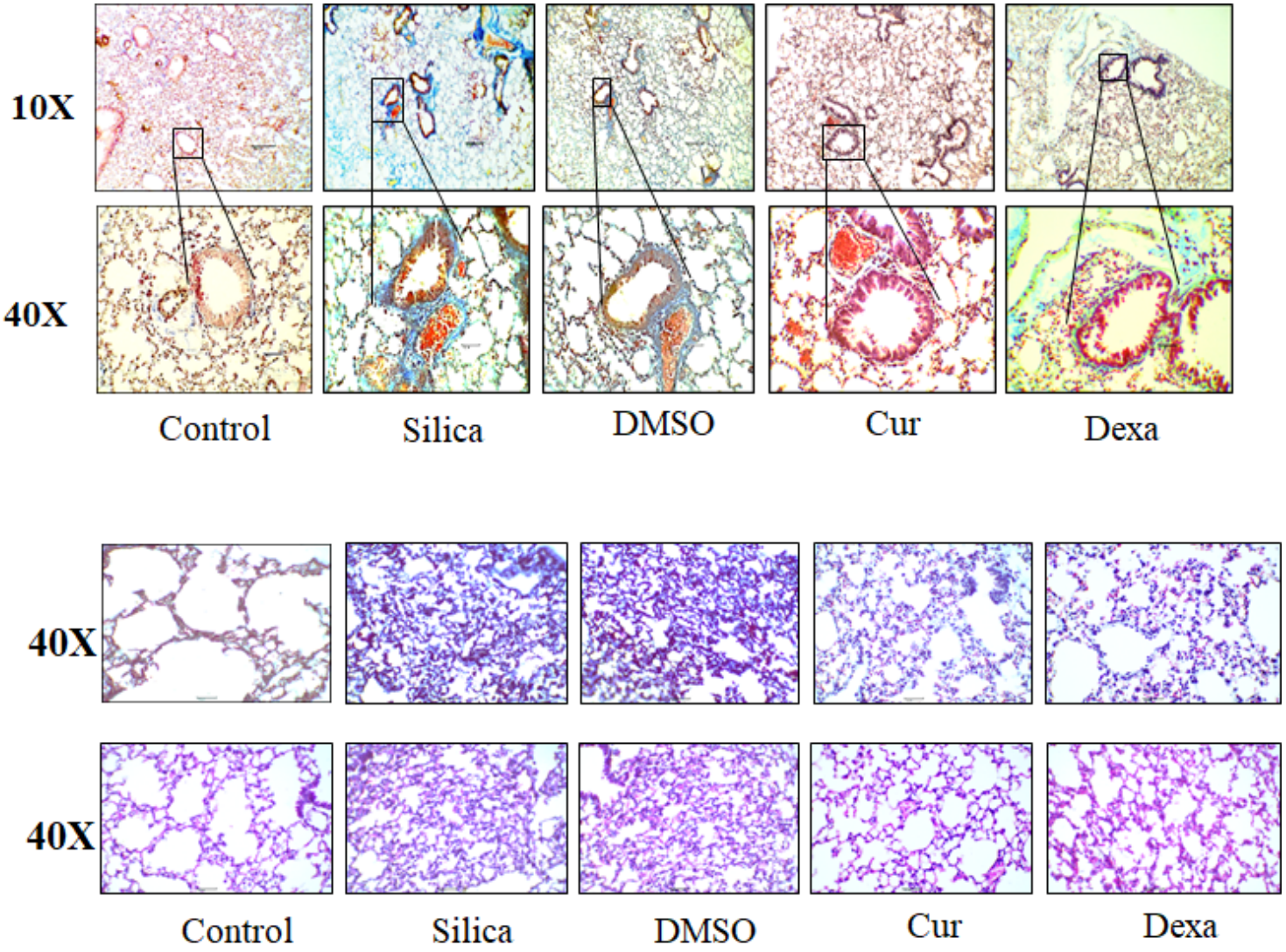

Fig 6

Figure 6

Masson’s trichome stained lung sections. Silica exposure and collagen deposition around bronchioles, blood capillaries and alveolar spaces (10X and 40 X magnification under light microscope).

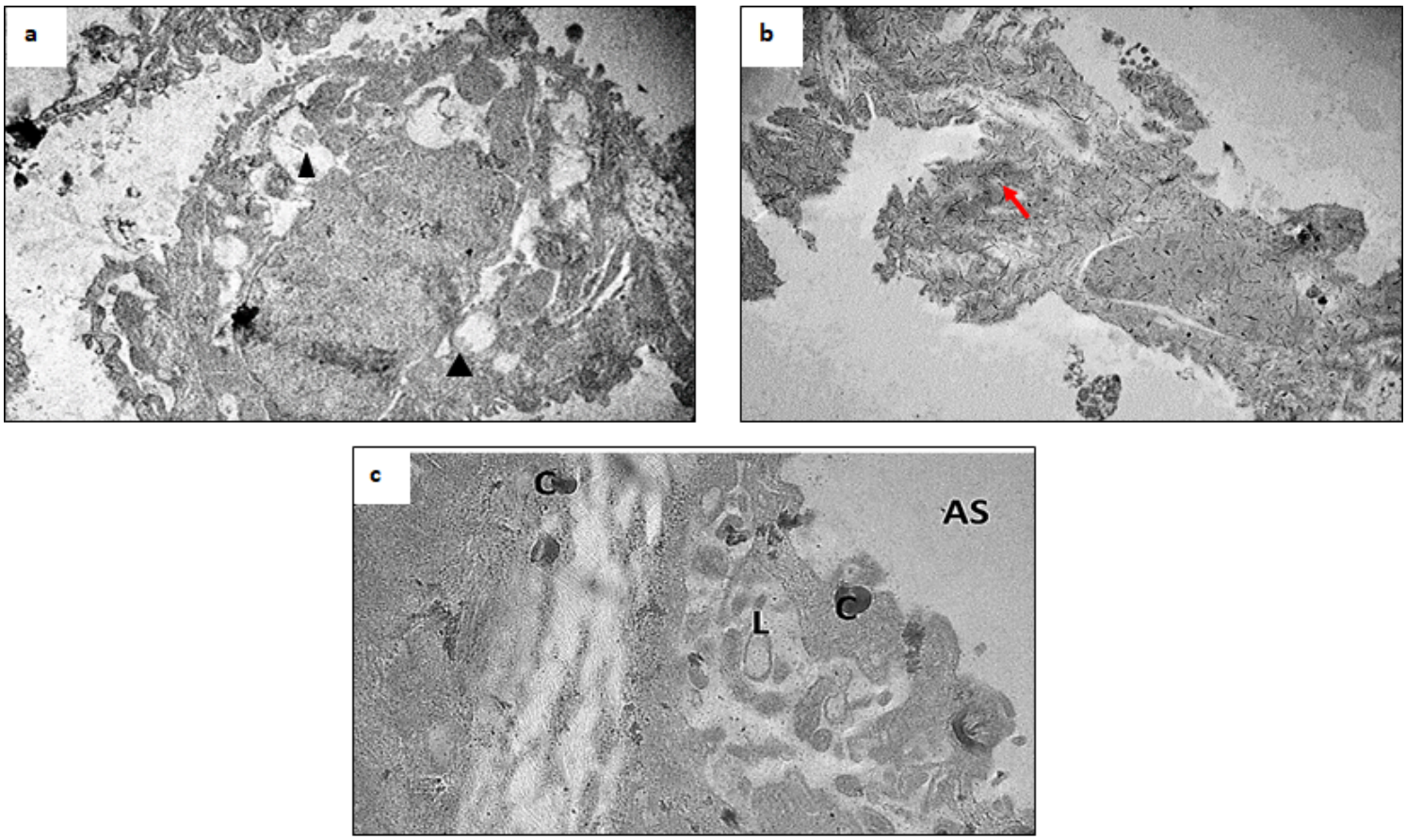

Fig 7

Figure 7

TEM image silicosis affected lungs showing (a) Haemozoin (arrow heads) inside the alveolar macrophages; (b) Localised areas around the alveolar epithelium were surrounded by collagen in silicosis group; (c) Oedema and ruptured blood vessels with thick alveolar walls which was full of congested capillaries and white blood cells (WBCs) AS, alveolar space; C, capillary; L, leukocyte. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

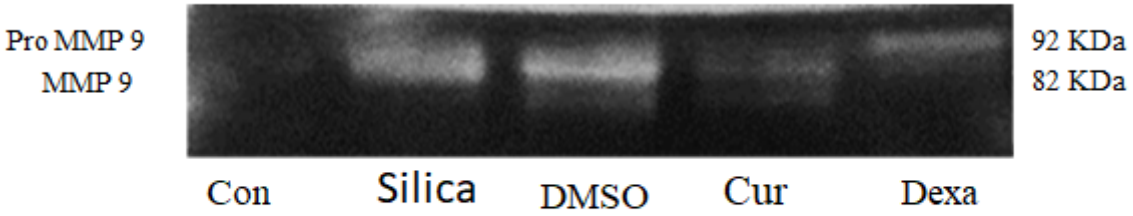

Fig 8

Figure 8

MMP-9 activity in BALF using gelatine zymography. Higher MMP-9 activity in silica group which was markedly decreased in curcumin treatment group.

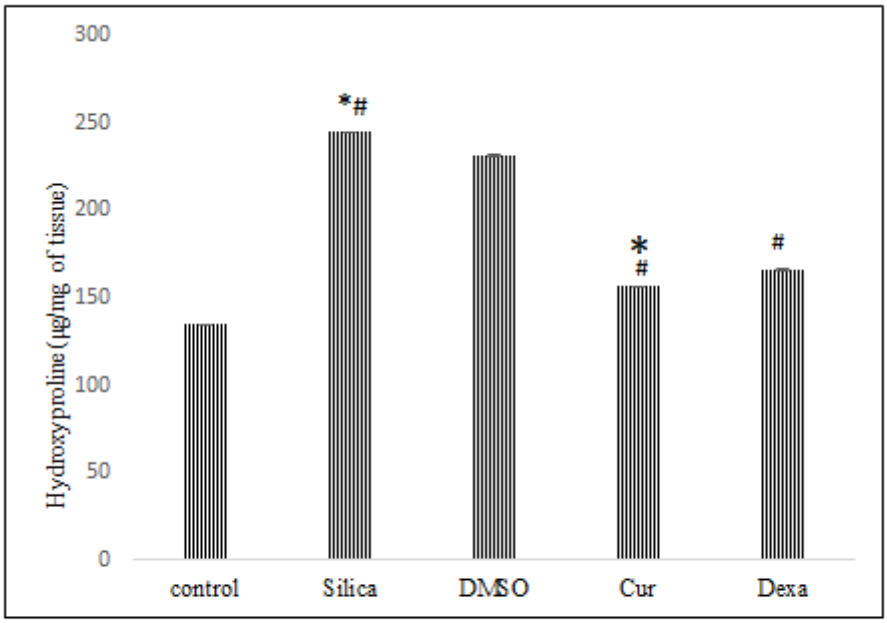

Fig 9

Figure 9

Hydroxyproline level in lungs. Silicosis group showed significantly higher level as compared to control ($p<0.05$) . Intra nasal curcumin pre-treated group #silica vs Curcumin group ($p<0.05$) (n = 5 in each group).

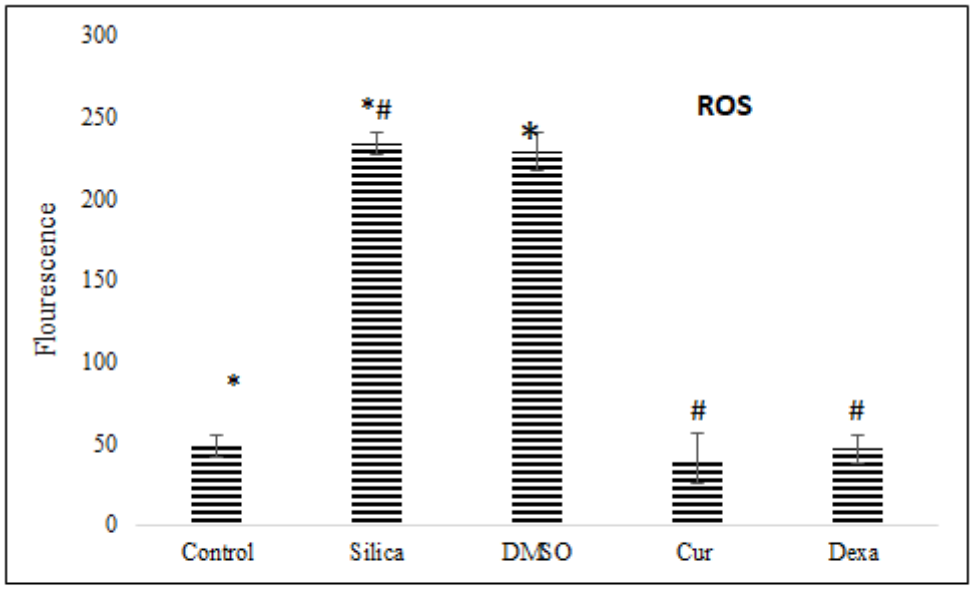

Fig 10

Figure 10

Reactive oxygen species (ROS) determination in BALF cells. Significant increase in ROS level was noted in Silica group as compared to control group whereas significant reduction after curcumin and dexamethasone treatment was noted. The value are ± SEM (n=5), control vs silica* pretreated group, silica vs i.n curcumin and i.p dexamethasone pretreated group#, $p<0.05$ (n=5).

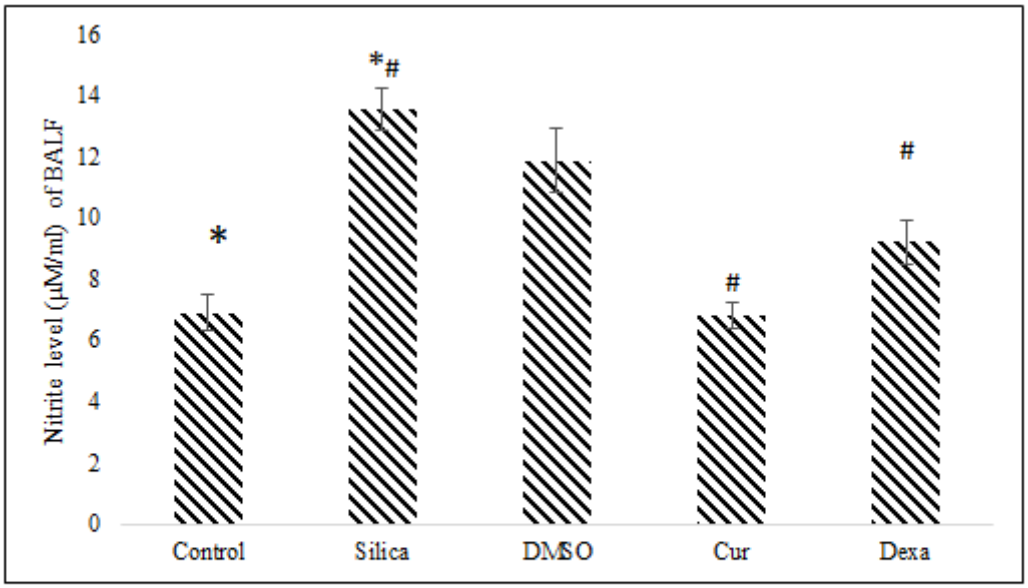

Fig 11

Figure 11

Nitrite level determination in BALF supernatant. Silica group showed significant increase in NO level as compared to saline treated control group. Curcumin and dexamethasone showed lower nitrite level than that of silica treated groups. The value are ± SEM (n=5), control vs silica* pretreated group, silica v s i.n curcumin and i.p dexamethasone pretreated group#, p<0.05 (n=5).

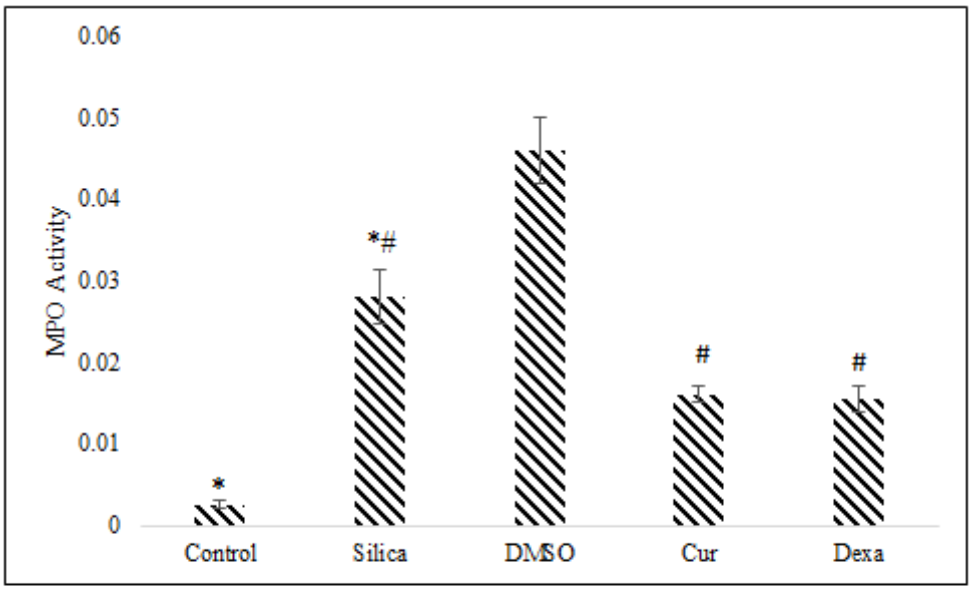

Fig 12

Figure 12

Myeloperoxidase (MPO) activity after silica exposure. Significantly higher MPO activity was noted in silica group which was reduced in intranasal curcumin and dexamethasone group. The value are ± SEM (n=5), control vs silica* pretreated group, silica vs i.n curcumin and i.p dexamethasone pretreated group#, p<0.05 (n=5).

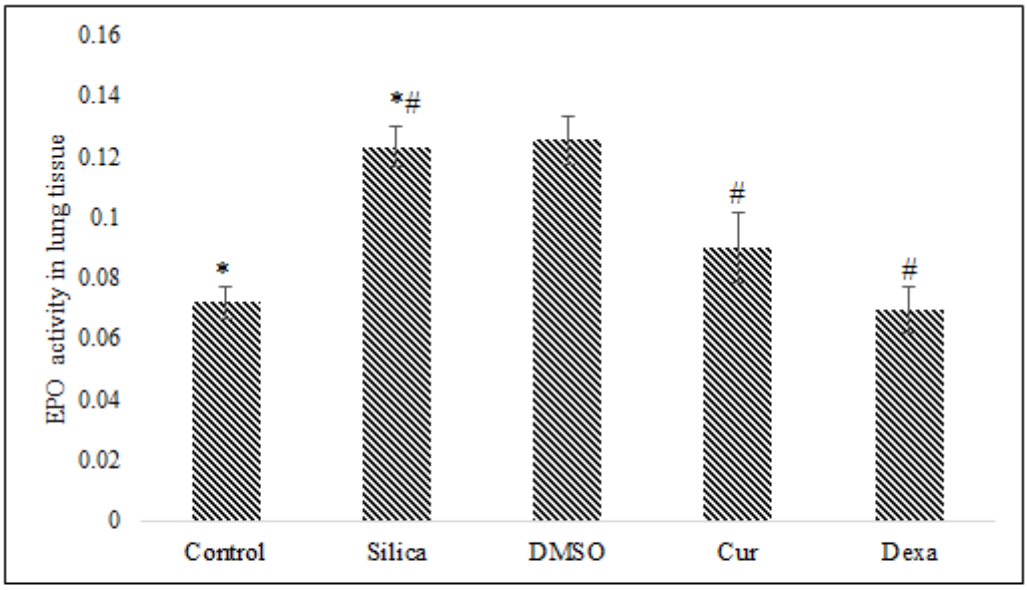

Fig 13

Figure 13

EPO activity in silica exposed lungs. Significantly higher EPO level was reduced in curcumin treatment group. The value are ± SEM (n=5)control vs intranasal silica* group vs curcumin + dexamethasone pretreated group# ,p<0.05

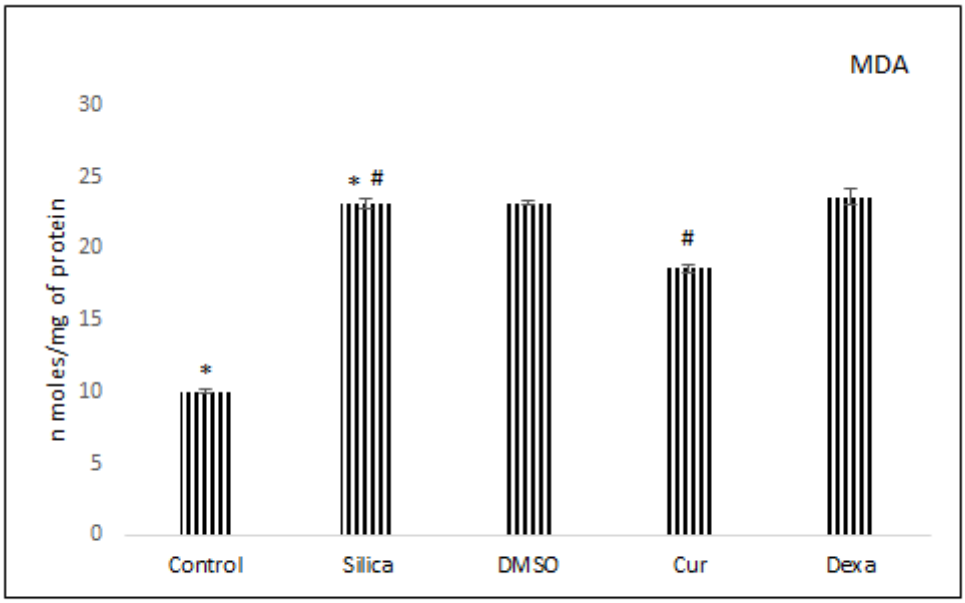

Fig 14

Figure 14

Malondialdehyde (MDA) is common marker for oxidative stress its level was reduced significantly in curcumin treated group than the silica group. #silica vs curcumin group ($p<0.05$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.pptx